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One-dimensional quasi-crystals of perfect multilayers, in which ion channels are uniformly oriented within parallel membranes, can be used to study the structural bases of channel conductivities. We have developed 1) the techniques for preparing such multilayer samples and 2) the spectroscopic methods (circular dichroism and x-ray diffraction) for extracting structural information from these samples. The sample variables include electric field, water content, ion concentrations, etc. We have observed conformation changes of alamethicin with water content, a result in favor of the barrel model (rather than the flip-flop model) for the channel. Our goal is to probe the conformation changes of the channels as we vary the sample variables, in order to elucidate the molecular mechanisms of voltage-gating.

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Introduction

Because of the difficulty in making single crystals of membrane ion channels in their native forms (suitable for x-ray diffraction), there is a lack of structural information for understanding their molecular mechanisms. We believe that, under the circumstances, one-dimensional (1D) quasi-crystals of perfect multilayers, in which channels are uniformly oriented within parallel membranes, can be used to provide some of the much needed structural data. In the past few years, we have developed 1) the techniques for preparing such multilayer samples and 2) the spectroscopic methods for extracting structural information from these samples. Our goal is to investigate the conformation changes occurring in the channels when they are subject to electric field or variations in chemical conditions, in order to elucidate the molecular mechanisms of voltage-gating. A sensible approach to this complicated problem is to study simple model channels such as alamethicin and melittin first. However, it is important to point out that our method is applicable to natural proteins; for example, we have applied our method to study cytochrome b from yeast complex III in another research project. In the following, we review our objectives and the progress we made last year.

Objectives

1. Preparing multilayer samples of gramicidin, alamethicin and melittin, and experimenting the variations of their chemical conditions.

2. Circular dichroism (CD) of multilayer samples to study the orientations of the α-helical sections in the channels.

3. X-ray scattering of multilayer samples to study the ion binding sites.

4. Normal incident neutron scattering of multilayer samples to study the channel distributions in membrane.

5. Electric field studies of multilayer samples to create the voltage-gating condition in the channels.

Accomplishments

1. Multilayer Samples -- We have by now successfully produced perfect multilayers of lipid-pepitde mixtures between two surfaces of fused silica, electrode (indium tin oxide) coated fused silica, mica and beryllium. Different substrata are used depending on the type of experiment. The thickness of multilayers can be varied between 1 and 100 μm. The sample variables include the peptide/lipid ratio, water content (15 to 40% of sample weight) and ion (e.g. Na^+, K^+, etc.) concentrations. The lipids used so far include dilauryl-, dimyristoyl-, dipalmitoyl-, and diphytanoyl-phosphatidylcholine (DLPC, DMPC, DPPC and DPhPC, respectively). These samples are free of smectic defects, transparent to light, and perfectly ordered in the direction perpendicular to the substrata surfaces (the mosaic spread ~ 0°) (for details see Huang and Olah, 1987 and Olah and Huang, 1988a).

2. CD -- Although a theory describing the dependence of CD on the orientation of an α-helix was known since the 1950's (Moffitt, 1956), experimentally it remained unproven until recently. The reasons are complicated and they are discussed in our recent papers (Olah and Huang, 1988a and 1988b). Because α-helices are somewhat flexible, only short (and hence straight) peptides can provide uniformly oriented α-helices. And in fact this condition has been achieved only in our perfectly aligned multilayer samples. We used the CD of alamethicin embedded in multilayers to prove the Moffitt theory and simultaneously established that the α-helical section of alamethicin was perpendicular to membrane under the condition we prepared the sample. A special technique was devised to measure CD of multilayers with light incident on the membranes at various tilted angles. Figure 1 shows an example of such measurement. The technique is very sensitive to the conformation changes of peptides. For example, Fig. 2 shows the changes of CD of alamethicin in DPhPC with hydration. As explained in the Fig. 2 caption, the results appear to be in favor of the barrel model (Hall, Vodyanoy, Balasubramamian and Marshall, 1984) rather than the dipole flip-flop model (Menestrina, Voges, Jung and Boheim, 1986) for the formation of the alamethicin channel. A more complete analysis is in progress.

3. 1-D X-ray Diffraction -- Since our multilayers are in fact one-dimensional (1-D), quasi-crystals (perfect ordering in the direction normal to the planes of membranes), their 1-D electron density profiles can be determined by x-ray diffraction. It is, however, not easy to unravel the peptide signals from the intense diffraction background due to lipid bilayers. Therefore, our effort has been concentrated on measuring the ion distribution profiles, including the locations of the ion binding sites in the channels, by using heavy metal ions such as cesium and thallium. Early on we had difficulties with the x-ray absorption by the materials used to support the multilayers. This problem has now been solved by using beryllium. The diffraciton of thallium ions in the gramicidin channels has been measured. Twelve Bragg peaks were recorded, amounting to 2-3 Å resolution. The analysis, including solving the phase problem, is in progress.

4. Normal Incident Neutron Scattering -- This experiment was designed to measure the 2-D protein distribution in the plane of membrane, which contains information about the protein-membrane interactions (Huang, 1986). Other research groups have attempted similar measurements of 2-D protein distributions by using vesicular samples and always found their signals masked by that of vesicles (despite their efforts of index matching with deuteration). On the contrary, the normal incident neutron scattering of defect-free, pure lipid multilayers gave a flat background (no angular dependence). Therefore if a protein has a reasonable neutron scattering contrast against the lipid background, its distribution can be measured. For this purpose, either peptide or lipid needs to be fully deuterated. We have placed an order with Avanti Polar Lipids (Pelham, AL) to synthesize fully deuterated DLPC. Their schedule and the problems in both the Oak Ridge and the Brookhaven neutron facilities have delayed the progress of this experiment.

5. Electric Field -- We have successfully coated indium tin oxide on fused silica surfaces so that an electric field of up to 50 kv can be applied across a multilayer sample (Olah and Huang, 1988b). The coated electrode is thin enough that it does not interfere with the CD measurement of the sample. However, the joule heating and the electrode damage at the anode have been problems. We have reduced the sample conductivities by purifying the chemical components. We have also coated the electrode with thin silicon dioxide to prolong its life. After many experiments, we found that we could apply to our samples electric field of square steps, each 0.1 s on and 0.9 s off, for hours. This allows at least two types of experiments. 1) Our CD spectrometer (Jasco J-500A) allows signal averaging with 0.1 s point measurements. Thus we can measure the CD of ion channels in electric field. 2) With synchrotron radiation, subnanosecond-resolved diffraction measurement is now possible (Science 241, 295, 1988). Thus we can measure the x-ray diffraction of ion channels in electric field.

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H. W. Huang, "Deformation Free Energy of Bilayer Membrane and Its Effect on Gramicidin Channel Lifetime" Biophys. J. 50, 1061-1071 (1986).

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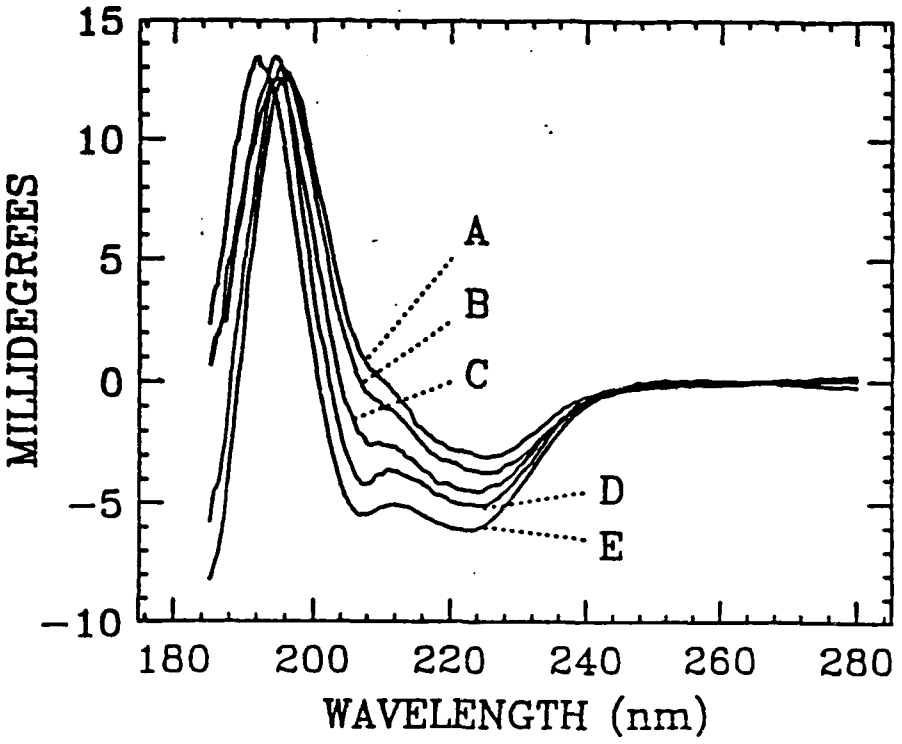

Fig. 1: Circular dichroism of oriented alamethicin embedded in DLPC multilayers at tilt angle (between the direction of light and the normal to the planes of bilayers) $\alpha = 0^{o}$(A), 15^{o}(B), 30^{o}(C) and 45^{o}(D). The amplitude at 208nm is proportional to $\sin^2\alpha$ as predicted by the Moffitt theory of α-helices; this is the first proof of the thoery (Olah and Huang, 1988a).

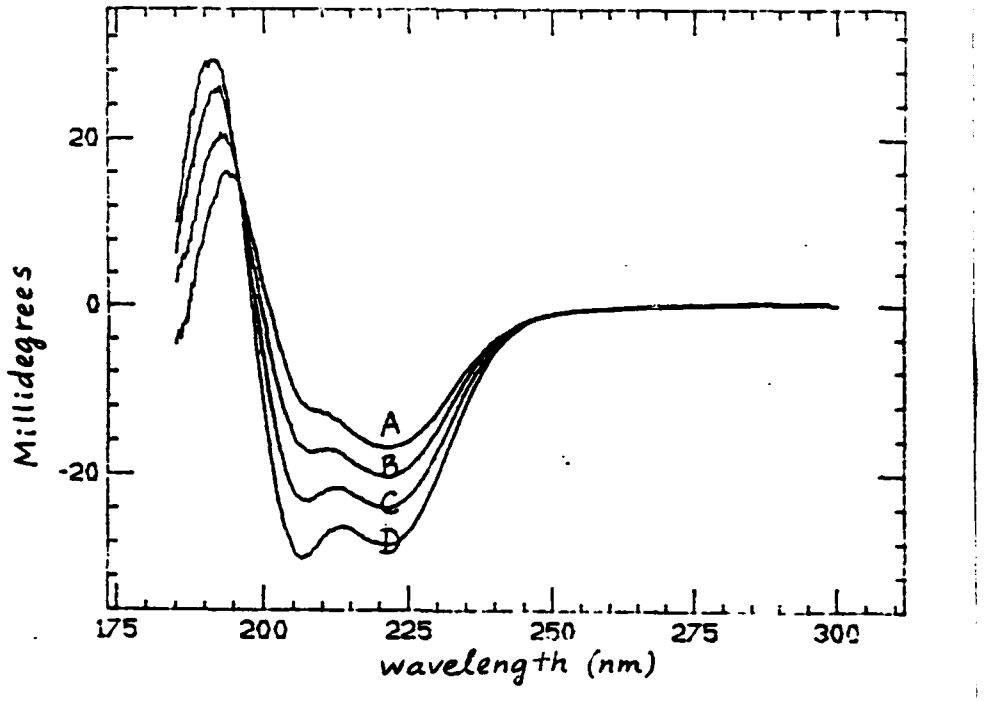

Fig. 2.: The changes of the circular dichroism of alamethicin embedded in DPhPC multilayers with the degree of hydration. Spectra were taken at normal incidence with the same sample by varying the equilibrating humidity: (A) 100% humidity; (D) 0% humidity; (B) and (C) in between. The results indicate that the insertion of α-helices into the membrane requires excessive water. This appears to be in favor of the barrel model (rather than dipole flip-flop model) for the formation of the alamethicin channel.

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